1969

The effect of blood from various vertebrate hosts on reproduction in Anopheles quadrimaculatus Say and Aedes aegypti (L.) : a comparative study of fecundity and viability.

Paul Albert King
University of Massachusetts Amherst

The Effect of Blood from Various Vertebrate Hosts on Reproduction in *Anopheles quadrimaculatus* Say and *Aedes aegypti* (L.): A Comparative Study of Fecundity and Viability

A Thesis Presented

by

Paul Albert King

Submitted to the Graduate School of the University of Massachusetts in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

June 1969

Major Subject Entomology

The Effect of Blood from Various Vertebrate Hosts on Reproduction in *Anopheles quadrimaculatus* Say and *Aedes aegypti* (L.): A Comparative Study of Fecundity and Viability.

A Thesis

by

Paul Albert King

Approved as to style and content by:

E. W. Lindsay

Frank K. Shaw

T. Michael Peters

Chairman, Head of Department

TABLE OF CONTENTS

DEDICATION

I am most appreciative of Dr. T. M. Peters for the many hours he has spent reading and critically evaluating this thesis as well as for his words of encouragement at moments of frustration. I am also thankful to Dr. Peters for his enthusiasm which provided motivation on my part to complete this endeavor.

I would like to thank Dr. Frank Shaw for having spent much time evaluating this thesis as well as providing me with several valuable references.

I am thankful to Dr. Ellsworth Wheeler who evaluated this work and Dr. Richard Damon who provided valuable statistical assistance.

I thank Mrs. Starzyk for her help in typing this thesis.

Last, but not least, I am grateful to my wife, Betsy, for her patience and help in putting this thesis together.

INTRODUCTION

Mosquitoes are among the most common insects inhabiting the earth. They are found not only in the tropics but also in the polar regions of the world. There have been 2,400 species described and with their tremendous ability to survive, multiply, and adapt, their numbers could increase. Mosquitoes may be found in any naturally standing water. Certain species are limited to either fresh, salt or polluted water. Man also offers homes for the mosquito by leaving water in tin cans, in tires and in homemade ponds.

Pest mosquitoes, in general, have been divided into five main categories based on their larval habitat. The categories are: (1) salt marsh, (2) fresh floodwater, (3) domestic, (4) permanent swamp, and (5) snow pool mosquitoes.

Mosquitoes are responsible for much of the discomfort brought to many warm-blooded animals as well as being responsible for the spread of organisms causing diseases such as malaria, yellow fever, dengue and encephalitis from which millions of people suffer. The discomfort caused by the mosquito is due largely to the bite of the female as she takes a blood meal from her host. This frequently produces severe itching and swelling in certain individuals. The male is not a blood feeder and does not bite. Because of these facts and

the ability to survive, the mosquito is of great economic and medical importance.

Perhaps the most important reason we are concerned with mosquitoes today is the fact that they aid in the spread of a variety of harmful diseases in parts of the United States. Malaria, one of the more commonly known diseases, is caused by the protozoan, Plasmodium sp., carried mainly by the genus Anopheles and passed to man by the bite of the mosquito. Prevalence of this disease is on the decline. However, it still affected six to seven million people in the 1930's in the continental United States. Before malaria can be completely eradicated, research and control programs must take into consideration not only the mosquito but the ecology of the disease and the victim himself.

Another prominent disease spread by a mosquito is yellow fever. Here the mosquito, primarily Aedes aegypti (L.), is infected with the virus when feeding on a diseased host, and it passes the organism when it bites another host. This disease has been practically eliminated in the United States in recent years, but is still of concern because of the possibility of jungle yellow fever, a similar disease in which monkeys are usually infected, becoming epidemic in man. Further concern arose over the possibility of new yellow fever outbreaks in North America, when the potential vector, Haemagogus equinus Theobold, was discovered in Texas. People doing research with this disease must investigate not only the mosquito vector, but also the potential hosts before this

disease can be completely eradicated.

The genus Culex may transmit certain forms of virus encephalitis, mainly a disease of domestic animals, but also occurring in epidemic form in man. Some examples of this type of disease are: St. Louis, Eastern, Western and California encephalitis. All of these occur in North America.

There are numerous research programs across the country to combat these diseases and the general annoyance of the mosquito. Many large companies in the United States and research centers at colleges and universities have put endless hours into the study of the mosquito with the hope that some day, pest mosquitoes and disease vectors will be completely controlled. Most of the present investigations of control methods fall into 4 types: (1) chemical, (2) water management, (3) biological control, and (4) individual protection.

All mosquitoes belong to the order Diptera, and family Culicidae in which there are three subfamilies, 2 of which are considered in this thesis. Aedes aegypti (L.) and Anopheles quadrimaculatus Say, because of their importance, their availability, short life cycle and ease in handling were used in the following study.

This experimental problem involved a comparison of the fecundity and egg viability of 2 mosquito species after feeding on 8 different hosts. I was interested in statistically determining whether significant differences exist in the nutritive value of the blood of poikilotherms versus homeotherms. If such a difference does exist it could account for differences

in numbers of eggs produced by Anopheles and Aedes after feeding on the 8 hosts. The differences among the 8 hosts in respect to fecundity and egg viability is of great interest since this may be of economic importance in respect to development of mosquito attractants. Knowledge of host acceptance could permit us to control host availability when suppressing mosquito populations. In the statistical methods employed, the 2 mosquitoes and the 8 hosts are considered the independent variables while fecundity and egg viability are the dependent variables.

LITERATURE REVIEW

Host Preference

Aedes aegypti (L.). Howard, Dyar, and Knab (1912) noted that certain species of mosquitoes have a decided preference for particular hosts. They contend that while human blood is preferred, some Aedes aegypti will also feed on birds and other mammals. Many Aedes spp. prefer mammalian blood (Edman & Downe 1964). Christophers (1960) reported the guinea pig and rabbit as satisfactory sources of blood meals in lab rearing of Aedes aegypti. Fielding (1919) tested the guinea pig and rabbit as hosts but found feeding on small animals less successful than on humans since nonhuman hosts had to be immobilized and shaved for good results. It should be noted that Fielding's results differ from those of other workers. With a proper restraining cage (Bishop & Gilchrist 1946) the chicken can be used as a host. In a recent study of host preference among a guinea pig and an eastern garter snake (Thamnophis sirtalis sirtalis) Pearson and Harrison (1967) observed that a guinea pig was more attractive than an eastern garter snake to Aedes aegypti (L.).

Cold-blooded vertebrates are often hosts for mosquitoes (Howard, Dyar, and Knab, 1912), therefore some attempts have been made to feed Aedes aegypti (L.) on cold-blooded vertebrates and invertebrates. Woke (1937 a) was successful in

getting Aedes aegypti to feed from a turtle (Terrapene carolina) and a frog (Rana clamitans). Gordon and Lumsden (1939) also observed Aedes sp. feeding on frogs' blood. In work with Aedes aegypti (L.) and Ae. albopictus Skuse, Toumanoff (1949) found that Aedes aegypti fed on reptiles were "weaker" than those fed on humans.

Nolan, Moussa and Hayes (1965) recorded females of Aedes canadensis (Theobald) and Aedes triseriatus (Say) feeding on turtles in nature. DeFoliart (1967) reported similar results with Aedes canadensis. Another example of Aedes aegypti feeding on a reptile is given by Pearson and Harrison (1967) in which several females engorged on an eastern garter snake. Warming a frog or turtle induces a significantly greater number of Aedes spp. to feed (Willis, 1958). This simulates the warm body temperature found in homeotherms and is probably responsible for attracting the mosquitoes.

As compared to mamalian hosts, the mosquito tends to take a smaller blood meal from the frog and turtle and yet lays a greater number of eggs per millimeter of blood ingested (Woke, 1937 b). A complete engorgement would occur when the mosquito fed on a human, but the number of eggs per millimeter of blood ingested would not be significantly greater. The number feeding depends on surface area of the host since Aedes spp. will select the larger of 2 animals in a field test (Downe, 1960). This is contradictory to results of Howard, Dyar, and Knab (1912) in which they contend that human blood is preferred by Aedes spp. over other animals.

Anopheles quadrimaculatus Say. Anopheles spp. prefer mammalian hosts, especially larger mammals (Howard, Dyar, and Knab, 1912). The responses of An. quadrimaculatus Say to various hosts are reported by Bull & Root (1923) and King & Bull (1923) in which they considered that humans attracted a smaller number of biting An. quadrimaculatus than either horses or cows. They found rabbits and chickens to be poor hosts even in the absence of other sources of blood. However, Horsfall (1955) reported that An. quadrimaculatus feeds on man, cow, horse, pig, sheep, dog, cat, and fowl in nature. He found preference order difficult to establish due to differences in collecting sites, yet humans tend to be chosen first, cattle and horses second, and sheep and cats last for mosquitoes caught in domestic situations. Reid (1961) substantiates previous reports that Anopheles spp. prefer mammalian hosts. He found the darkwinged form of An. barbirostris Wulp favoring man and An. vagus Don favoring calf over man. Murphy et al. (1967) showed in their experiments that all their traps baited with homeotherms contained engorged females of An. quadrimaculatus Say. They also found a few of the mosquitoes in traps baited with reptiles. They concluded that An. quadrimaculatus feeds predominantly on mammalian hosts with only a moderate attraction to avian hosts and limited feeding on a reptilian host. This feeding on a reptile had not previously been reported. Willis (1958) was able to induce small numbers of An. quadrimaculatus to feed on a warmed frog and toad. They did not respond to a lizard even when warmed.

Smith (1955) tested reactions of An. gambiae Giles to small animals in cages. He found that this mosquito would feed on the African bullfrog (Rana occipitalis), but not on reptiles. However, the chief source of blood for this species, according to precipitin tests, is man and ox. This is also true for An. funestus Giles.

An interesting theory proposed by Roubaud in 1920 assumes that in certain mosquito species such as Anopheles maculipennis, a biological differentiation has occurred producing 2 "physiological races"; one prefers humans and the other prefers larger mammals as a source of blood meals. He suggested that a gradual adaptation to feeding on domestic animals, in areas where animals are stabled for extended periods of time, has brought about this biological differentiation of a race of Anopheles maculipennis which is larger than normal (Wardle, 1929). This An. maculipennis complex has been divided into a number of species and subspecies which are not well understood. Bates (1940) lists 5 species and 2 subspecies. These are An. maculipennis Meig, the typical form widely distributed in Europe, An. messeae Falleroni, An. melanoon melanoon Hackett, An. melanoon subalpinus Hackett and Lewis, An. labranchiae labranchiae Falleroni, An. labranchiae atroparvus van Thiel, and An. sacharovi Favr.

Factors influencing feeding stimuli of female mosquitoes. Age of the mosquito, time of day, and atmospheric temperature (Horsfall, 1955), as well as wind, rain and the

time interval since the mosquito last fed affect feeding by mosquitoes.

A female Aedes aegypti (L.) seeks a host through responses to chemical and visual stimulation. These mosquitoes fly upwind toward odors emanating from humans or their excretory products. Once the mosquito enters an environment permeated by human odor, visual stimuli become important with the orientation in the direction of a line separating light and shadow (Kennedy, 1939). The investigations of Brown et al. (1951) revealed that moisture and color of surfaces affect the landing rate of hungry caged female Aedes spp. Parker (1948) found that mosquitoes were attracted more to sweat than to moisture alone. On the contrary, Bates (1949) was not able to trap mosquitoes when using sweat as bait. His results agree with those of van Thiel (1937) in which the sweat and blood of man or pig would not attract any mosquitoes. Van Thiel did observe that carbon dioxide was strongly attractive to mosquitoes. Air with a relative humidity of 85 per cent attracted 3-5 times as many mosquitoes as air with a 15 per cent relative humidity (Brown et al., 1951). The heat of convection and the consequent turbulence in the vicinity of the body proves attractive to Aedes spp. A warm object (37° C.) was more attractive than a cold one (27° C.) (Peterson & Brown, 1951). Lumsden (1947) found a high biting rate in Aedes aegypti (L.) at 25-35° C. but few bit at a temperature of 15° C. He also found that relative humidity had no effect on biting activity over the range 5-98

per cent.

The biting activity of mosquitoes in aggregations was studied by Terzian and Stahler (1949). They found that the lowest biting rates occurred in those groups with the lowest percentage of males. With an increase in the percentage of males, there was an increase in the biting rate while the highest biting rates occur among those groups with the highest proportion of males.

The antennae are important in host location. All flagellar segments of female Aedes spp. and Anopheles spp. bear thin walled chemosensory hairs. These seem to be responsible for sensitivity of the females to odor, CO_2, and possibly temperature (Roth, 1951).

Many workers have conducted extensive research on the responses of adult female Ae. aegypti. Peterson and Brown (1951) studied the fact that warm bodies are more attractive than cool ones irrespective of their humidity and found this a positive factor in host attraction. Emission of 10 per cent CO_2 from the head of a heated dummy increased the landing rate by 30-60 per cent over a control from which no gas was emitted (Brown, 1951). When the host is stationary, airborne factors are more important, but in a moving host visual factors are more powerful (Sippell & Brown, 1953). Smart and Brown (1956) found that darker colors attract significantly greater numbers of Ae. aegypti. Clements (1963) concluded that mosquitoes orient towards currents of moist air and show a strong tendency to alight at the source provided humidity

is not raised to near saturation. It appears that an air current containing CO_2 is sufficient to cause activation, orientation, and alighting of the mosquito, but in the presence of a second factor such as heat, the activated mosquito will orient to that factor (Clements, 1963).

Heat acts as a stimulus for a positive taxis while CO_2 and odors act only as stimuli for kineses in the orientation mechanism. Nuttall and Shipley (1902) reported An. maculipennis readily attracted to any dark objects. The use of dark clothes by workers to capture Anopheles spp. in enclosures has been mentioned often in the literature.

Laarman (1958) concluded that internal factors in the mosquito cause it to react to host stimuli. The feeding drive develops gradually and is independent of fertilization. He concluded that higher humidity is not a factor in host selection. He believes that contact by repeated generations with one host species may create a host preference based on specific odors. A similar preference mechanism has been shown in some Lepidopterous species (Bates, 1965).

In a newly emerged laboratory colony of Anopheles quadrimaculatus Say, the biting drive builds up slowly. The number ready to bite increases steadily from 18 hours after emergence until by 48-60 hours 90 per cent usually have engorged (Burgess & Young, 1944; Keener, 1945).

Fecundity. Fecundity of Aedes aegypti (L.) has been quite thoroughly studied by many workers. Mathis (1934) reported that human blood enables Ae. aegypti to produce about

one-fourth more eggs than monkey, rabbit, or guinea pig blood. Woke (1937 b) found that Ae. aegypti laid more eggs per mg. of blood ingested from rabbit or guinea pig than from canary or turtle. Ae. aegypti (L.) was believed to prefer man, yet Wcke concluded that human blood is lower in nutritive value than that of other mammals or reptiles used in his tests.

Number of eggs produced varies not only according to the quantity of blood ingested, but also according to reproductive capacity of the female and nutritive value of the ingested fluid (Woke, 1937 b).

Most mosquitoes fed on frog and turtle produce viable eggs. Larvae and adults developed normally. This demonstrated that under certain laboratory conditions, Ae. aegypti (L.) can feed readily on frog and turtle and produce viable eggs (Woke, 1937 a). Toumanoff (1949) found that when Ae. aegypti (L.) and Ae. albopictus Skuse fed on the caterpillar Sericaria mori all died the following day without producing eggs. These Aedes spp. produced more eggs when they were fed human blood than when fed lizard's blood. As a result, Toumanoff (1949) stated that for these species, blood from poikilotherms is less favorable than human. These spp. have a shorter life span when fed on cold-blooded hosts. Woke's (1937 a) results are based on a limited number of blood feedings while Toumanoff's (1949) results are based on the entire life span of each Aedes spp. mosquito. This may account for their contradictory statement. Pearson and Harrison (1967) observed feeding by Ae. aegypti on a snake and a guinea pig and recorded a time of development

for the subsequent generation, yet no comparison was made of the two hosts with respect to fecundity. The fecundity of Aedes aegypti (L.) is not significantly affected when fed on humans as compared to lab animals (Pena de Grimaldo & LaVoipierre, 1960). Willis (1958) fed Ae. aegypti and An. quadrimaculatus on an anuran. Both mosquitoes produced young that hatched and developed normally. Ae. aegypti fed on a lizard did not oviposit.

Few workers have conducted research on the fecundity of Anophelines. Anopheles maculipennis fed on human blood produced many eggs (Roubaud, 1934), yet Pena de Grimaldo & LaVoipierre (1960) confirm previous reports that many mosquitoes will lay more eggs capable of development after blood feeding on lab animals than after feeding on man. These latter workers based their statement on Aedes aegypti. Willis (1958) managed to get some An. quadrimaculatus females to feed on a warmed frog and lay viable eggs, but he could not induce their feeding on a lizard.

Volume of the blood meal in mosquitoes may vary from 2.2 mg. to 4.0 mg. (Horsfall, 1955). Mayne (1928) found the weight of an average meal among Anophelines to be 3.0 mg. or an amount equal to that of the mosquito's unengorged weight. Geoffrey (1956) recorded Anopheles quadrimaculatus Say ingesting an average of 3.46 mg. of mammalian blood; 1-1/2 times the mean unfed weight of the mosquito. Woodward and Chapman (1965) showed that An. quadrimaculatus more than tripled its body weight after blood feeding.

The number of eggs laid after a single blood meal by Aedes aegypti (L.) correlated with amount of blood ingested only when blood meal was medium sized or smaller. There is no increase in egg production by Ae. aegypti after 3 mg. of blood have been ingested (Woke et al., 1956; Colless & Chelapah, 1960). Clements (1963) on the other hand, contends that the size of the blood meal does not affect fecundity in Ae. aegypti unless it falls below about 2 mg. The number of eggs laid shows a positive correlation with the size of the female in several species of Anophelines (Roy, 1931; Shannon & Hadjinicalao, 1941).

MATERIALS AND METHODS

Anopheles quadrimaculatus Say. The Anopheles culture at the Department of Entomology, University of Massachusetts, was received from the Harvard Medical School in Brookline, Massachusetts, during the Fall of 1967. The following is a brief description of the rearing techniques used to maintain the culture and to produce experimental animals.

The female deposits her eggs on the surface of distilled water and moist paper toweling which line the inside of a 13-1/2 x 9-1/2 x 2-1/8 white porcelain pan containing 300 ml. of distilled H_2O and placed inside the culture cage. The pans are changed every other day. Eggs are removed from the paper toweling by holding it upright over the pan and washing the eggs off with water from a squeeze bottle. From this pan, the first instar larvae can be counted as they are expelled from a medicine dropper into a pan of distilled water.

The larval developmental period lasted approximately 19 days under the following conditions: Larval pans were kept in a growth chamber with a 12-hour light-dark period, air temperature of 78±2°F., and relative humidity of 78-80 per cent. First instar larvae are placed in porcelain pans containing 500 ml. of distilled water. Six hundred larvae were placed in each pan designated for use with a specific host. These larvae were fed twice daily approximately 0.004 g.

of dried Brewer's yeast which had previously been sieved through a 140-mesh screen. The feedings were approximately 6 hours apart each day. On day 5, feeding was increased to 0.049± g/pan. This level of food was maintained until pupation. Food was applied to the water's surface and could easily be seen in the surface film.

The food measuring device for the initial feeding period was made from a 6-inch piece of glass tubing with a 2.5 mm. inside diameter, the end of which was bent at a right angle. A piece of cork was inserted 1 mm. into this end of the tubing. The longer end of the tubing was used as a handle and allowed easy access into the yeast jar. The tool used to apply food after the first 5 days was similarly fashioned from a piece of 4 mm. inner diameter glass tubing. The cork in this measure was placed at a depth of 4 mm. Both measuring devices were adequate in measuring a relatively constant amount of food for the larvae as shown in Table 12. Care was taken to remove excess yeast adhering to the tubing as it was being filled by a slight tap of the applicator on the jar before placing it over the larval pan. The yeast was easily removed from the applicator by a slight tapping of the handle on the edge of the porcelain pan.

The pans were cleaned 9 times during larval development by syringing out most of the water then adding distilled water to replace that removed.

The mortality of the larvae during rearing was approximately 40 through 50 per cent with the most mortality occurring

in instars 1 and 4.

Pupation occurred about 19 days after egg hatch. Pupae were picked up with a blunt end medicine dropper and placed in open petri dishes half filled with water. A cone made from number 1 Whatman filter paper was then placed in each petri dish to facilitate emergence of adults. These pupal dishes were then placed in 18" x 9" x 9-1/4" aluminum screen cages, which had been previously autoclaved. These cages with pupae were maintained in the growth chamber.

The adults emerged 2-3 days following pupation, males usually emerging prior to the females. Mating occurred shortly after the females emerged, either in the early evening or in the very early morning. As soon as adults began to emerge, a slice of apple and a vial containing 10 per cent sucrose solution with a cotton wick were placed in each cage to provide food for the adults. A host was placed in the cage 6 days after the introduction of the pupae. This allowed for maximum emergence, fertilization and feeding. All hosts were introduced into respective cages through a cotton sleeve at one end. They were restrained, except for the turtle, during the blood feeding. The mammals, enclosed in a wire mesh, all had shaven backs to provide easier access to skin. The birds used were held to a board by elastic bands and the feathers removed from one thigh and leg. The turtle was warmed in an aquarium containing 86°F. water for 15 minutes then placed on its back in the cage. It soon exposed its neck and legs which were attractive to some Anophelines. The frog was placed in

95°F. water for 15 minutes then was restrained in a small screen cage. Three females engorged on the heated frog while none fed on the cold or unrestrained frog.

The engorged females were removed singly in vials and each placed in a round half-pint paper carton covered with a glass petri dish. A 60 mm. petri dish containing 2 ml. of distilled water was placed in each container. Water was applied with a hypodermic syringe. Two toothpicks were also placed crosswise in these small petri dishes to facilitate the exit of the female from the water and reduce the possibility of drowning. All containers were placed inside the growth chamber. In 2-3 days the fertile engorged females laid their eggs on the water in the petri dishes. The adults were then returned to the main culture cage. The eggs were counted, then 2 ml. of distilled water were added to the dishes to prevent drying. Two to 3 days later larvae hatching from these eggs were counted as they were expelled from a pipette into a pan.

Aedes aegypti (L.). Aedes aegypti (L.) used were originally obtained from a laboratory colony at Rutgers University and are currently maintained at the University of Massachusetts.

The eggs of Aedes aegypti are stored on filter paper cones in a desiccating jar. A piece of one paper cone containing several hundred eggs was placed with the eggs facing down in a petri dish half filled with water. The dish was placed in a vacuum jar. Vacuum at a pressure of 20 psi. for

30 min. initiated hatching. Five hundred 1st instar larvae were counted out for each porcelain pan used.

Each porcelain pan, 13-1/2" x 9-1/2" x 2-1/8", contained 2000 ml. of distilled water plus the 500 larvae. One pan of 500 larvae was designated for each host so as to be certain of enough adults for each host. The larvae were fed according to a feeding schedule designed by Dr. T. M. Peters and Mr. Boris I. Chevone of the Entomology Department at the University of Massachusetts. The feeding schedule is based on number of larvae in a given amount of water. The larval period lasted 7 days. The larval food, dried Brewer's yeast, was given in the following amounts for each consecutive day: .425 g.; .575 g.; .725 g.; 1.175 g.; .825 g.; .825 g. The food was applied in suspension rather than as a powder on the water surface. The larval period normally lasts only 5 days according to this schedule, but since the water in the pans was not changed daily, the larval period was lengthened by 2 days. The pans were cleaned of food residue and larval excretory products on days 2, 4, 5, and 6. These pans were maintained in the growth chamber under conditions given in the Anopheles quadrimaculatus rearing procedure.

The larvae pupated on day 8 after egg hatch and were placed in open petri dishes. Handling of Aedes aegypti pupae was similar to that method mentioned in the Anopheles quadrimaculatus section. Petri dishes were then placed in the aluminum screen cages. Two days were allowed for all adults to emerge.

As adults accumulated in the cages, a 10 per cent sucrose solution was placed on the floor of each cage in a vial containing a cotton wick. These cages were maintained in the growth chamber for 4 days after pupation to ensure emergence and adequate mating. Each host was introduced into a cage. Blood-fed females were collected in separate 10 dram vials and covered with a cotton plug. The vials were placed in a rack which was positioned to hold the tubes at a 45° angle and placed in the growth chamber. The day following the blood meal, a 2" x 1/2" piece of filter paper was placed in each vial. This was accomplished by raising one edge of the cotton plug and sliding the paper into the vial without disturbing the mosquito. This paper was moistened by using a hypodermic needle and passing the needle through the cotton plug and injecting 2 ml. of water onto the paper. This allowed for adequate moisture in the tubes and provided the females with an oviposition site.

Eggs were laid on the second and third day following blood feeding. The eggs tended to be deposited in greater numbers along the bottom and two sides of the paper. These were counted through the vial using a binocular microscope.

The filter paper was kept moist for the next 3 days by injecting 2 ml. of distilled water on the second day to maintain moisture. On the third day, 20 ml. of distilled water were poured into each vial. This drowned any remaining adults and induced hatching of the eggs. Larval counts were taken on the fourth day following the flooding of the vials.

The larvae were collected in a medicine dropper and counted as they were expelled into a pan of water. The larvae were later destroyed.

HOST ANIMALS USED

All the hosts used were apparently healthy vertebrates. The rabbit (Oryctolagus cuniculus), guinea pig (Cavia cutleri), and hamster (Cricetus frumentarius) were all obtained from the Department of Veterinary and Animal Science at the University of Massachusetts. These mammals were fed Blue Seal rabbit pellets. The human host in these experiments was myself. The chickens (Gallus domesticus) were obtained from the Tilson experiment farm, a branch of the University and were fed Blue Seal growing crumbles. The quail (Coturnix japonica) was obtained from a culture maintained by the Department of Forestry and Wildlife Management at the University and was also fed crumbles. The turtle (Terrapene carolina) was obtained from a local biological supply house. This host was kept in a partly filled aquarium and fed live earthworms. The frog (Rana pipiens) was obtained from a pond in Hadley, Massachusetts, and was kept in a partly filled aquarium and fed live American cockroaches.

ANALYSIS OF DATA

After completion of the experimental work with Aedes aegypti and Anopheles quadrimaculatus, the information gathered was arranged in tables. Tables 7 through 11 (Appendix) indicate the number of eggs laid and the per cent hatched from Aedes aegypti and Anopheles quadrimaculatus females that fed on each of the 8 hosts. A computer program was used to interpret these data. The program was arranged to analyze results obtained when the number of observations differed with respect to number of mosquitoes that fed on each individual host. The data in Tables 1 and 2 were derived from the raw data in Tables 7 through 11. Table 1 shows an analysis of variance on the number of eggs laid by Aedes aegypti and Anopheles quadrimaculatus after each mosquito blood fed on specific hosts. The data in Table 6 indicate whether there are any statistically significant differences between each independent variable which are the hosts and mosquitoes as well as their interaction and one of the dependent variables, namely the number of eggs laid. Table 2 shows an analysis of variance on the per cent hatch of eggs laid by these mosquitoes after feeding on the 8 hosts. This table also is an analysis of variance similar to Table 1 except that the other dependent variable, per cent of eggs hatched, is analyzed against the independent variables; the 2 mosquitoes and 8 hosts. Tables

3 through 6 involve Duncan's Multiple Range Test for unequal numbers. In Table 3 each host is compared in respect to the mean number of eggs Anopheles quadrimaculatus laid after feeding on each of the hosts. Table 4 presents similar information for Ae. aegypti. Tables 5 and 6 are host comparisons in respect to the number of eggs that hatched from An. quadrimaculatus and Ae. aegypti respectively after blood feeding on these individual hosts. Duncan's Multiple Range Test was performed using the treatment means as data, and indicates whether or not there are any statistically significant differences among the 8 hosts. The data in Table 12 represent attempts to arrive at a standard amount of dried Brewer's yeast to feed the An. quadrimaculatus larvae as they mature.

RESULTS

The analysis of variance of the number of eggs laid by Aedes aegypti and Anopheles quadrimaculatus females as shown in Table 1 indicates that there is a significant difference between the mosquito species and the number of eggs produced after each species fed on the 8 individual hosts. Aedes aegypti consistently produced more eggs after blood feeding on any of the 8 hosts than did Anopheles quadrimaculatus. The only instance in which An. quadrimaculatus had a higher mean fecundity than Ae. aegypti was when An. quadrimaculatus blood fed on the rabbit (see Table 3). Table 2 shows there is no significant difference between An. quadrimaculatus and Ae. aegypti in regard to the viability of eggs produced from females fed on any of the 8 vertebrate hosts.

There is a significant difference between the 8 hosts when fecundity of each species is used as the criterion (Table 1). In comparing the 8 hosts as to fecundity of An. quadrimaculatus and Ae. aegypti, Duncan's Multiple Range Test shows there is little similarity in results; although for both species, the rabbit proved a better host than the human.

The interaction of the 8 hosts and 2 mosquito species shows a significant difference between the fecundity of the females and egg viability after feeding on each of these hosts (Table 1). The results indicate that An. quadrimaculatus laid

the greatest number of eggs after feeding on the rabbit in comparison to the other 7 hosts (Table 8). This host was significantly different from all other hosts. Table 3 also shows that of the 8 hosts, An. quadrimaculatus produced the least number of eggs after feeding on the human. The other 7 hosts were significantly different from the human regarding eggs laid. The turtle, frog and guinea pig were not significantly different from each other and of these three hosts, the guinea pig was the only one different from the chicken, hamster, quail and human. The frog, turtle, chicken, hamster and quail were not significantly different from each other as hosts in regard to fecundity of An. quadrimaculatus.

When Ae. aegypti fed on these same hosts and a range test was performed to determine differences among them using subsequent fecundity as a criterion, the turtle, frog and human were not significantly different from each other yet were different from all other hosts. There was no significant difference between the chicken and quail regarding the number of eggs laid by Ae. aegypti after feeding on them, yet the mean number of eggs laid by Ae. aegypti after feeding on the birds was higher than on the other hosts. The quail was not significantly different from the chicken with respect to the number of eggs laid by Ae. aegypti after feeding on these two hosts, yet it was from all other hosts. The chicken was not significantly different from the rabbit, guinea pig, or hamster yet it was from the turtle, frog and human when Ae. aegypti females fed on these hosts. Ae. aegypti produced the fewest

number of eggs when fed on the frog.

The mean number of An. quadrimaculatus eggs that hatched after the adult female fed on the rabbit was significanuly different from egg hatches after feeding on the other 7 hosts. Egg hatch following the feeding by An. quadrimaculatus on the rabbit was significantly greater than with any of the other 7 hosts. The guinea pig and turtle were not significantly different from each other yet the guinea pig was significantly different from quail, frog, chicken, hamster and human in regard to Anopheline egg viability after feeding on these hosts. The turtle was not significantly different from the quail, frog, or chicken, but was from the hamster and human when An. quadrimaculatus fed on these hosts. Number of An. quadrimaculatus eggs which hatched after the adult female fed on the frog, chicken, hamster, or human were not significantly different from each other. The 2 poikilotherms were not significantly different from each other in this respect. The fewest number hatched when produced by An. quadrimaculatus females fed on the human host.

When Ae. aegypti fed on the 8 hosts and produced eggs, the resulting viability showed that the quail and guinea pig were not significantly different from each other, yet the quail was significantly different from all the remaining 6 hosts. The guinea pig was not a significantly different host from the chicken, hamster, or rabbit, but was different from the human, frog and turtle when we analyzed eggs hatched after

the Ae. aegypti females fed on these hosts. Egg hatch of Ae. aegypti is not significantly different when using the hamster, rabbit, human, frog and turtle as hosts. The greatest number of Ae. aegypti eggs hatched when they had been produced by a female fed on the quail and the lowest number hatched when the turtle was used as a host. The human proved to be a poor host for both An. quadrimaculatus and Ae. aegypti in regard to egg viability while the guinea pig was one of the better hosts for both of these mosquitoes.

TABLE 1.--Analysis of variance on number of eggs laid by *Aedes aegypti* and *Anopheles quadrimaculatus* when fed on various vertebrate hosts

Source of Variation	d.f.	Sum of Sqs.	Mean Sq.	F.
Mosquito	1	11898.545	11898.545	13.623**
Host	7	109055.821	15579.403	17.837**
Interaction (Mosquito-Host)	7	68577.580	9796.797	11.217**
Error	288	251542.730	873.412	

**Values significant at the 99% confidence level.

TABLE 2.--Analysis of variance on % hatch of eggs laid by *Aedes aegypti* and *Anopheles quadrimaculatus* when fed on various vertebrate hosts

Source of Variation	d.f.	Sum of Sqs.	Mean Sq.	F.
Mosquito	1	182.849	182.849	0.236
Host	7	19206.452	2743.779	3.547**
Interaction (Mosquito-Host)	7	25204.811	3600.687	4.655**
Error	288	222752.983	773.448	

**Values significant at the 99% confidence level.

TABLE 3.--Duncan's Multiple Range Test for Unequal Numbers on Fecundity of *Anopheles quadrimaculatus* when blood fed on 8 vertebrate hosts[a]

Human	Quail	Hamster	Chicken	Turtle	Frog[b]	Guinea pig	Rabbit
27	64	64	65	78	83	100	140

[a]The mean values connected by a line are not significantly different and those values not connected by a line are significantly different.

[b]Based on two observations.

TABLE 4.--Duncan's Multiple Range Test for Unequal Numbers on Fecundity of *Aedes aegypti* when blood fed on 8 vertebrate hosts[a]

Frog	Human	Turtle	Hamster	Guinea pig	Rabbit	Chicken	Quail
70	75	76	99	99	104	111	122

[a]The mean values connected by a line are not significantly different and those values not connected by a line are significantly different.

TABLE 5.--Duncan's Multiple Range Test for Unequal Numbers on egg viability of *Anopheles quadrimaculatus* when blood fed on 8 vertebrate hosts[a]

Human	Hamster	Chicken	Frog[b]	Quail	Turtle	Guinea pig	Rabbit
17	39	45	50	53	68	85	122

[a]The mean values connected by a line are not significantly different while those mean values not connected by a line are significantly different.

[b]Based on 2 observations.

TABLE 6.--Duncan's Multiple Range Test for Unequal Numbers on egg viability of *Aedes aegypti* when blood fed on 8 vertebrate hosts[a]

Turtle	Frog	Human	Rabbit	Hamster	Chicken	Guinea pig	Quail
54	55	59	70	73	78	88	92

[a]The mean values connected by a line are not significantly different while those mean values not connected by a line are significantly different.

DISCUSSION

Aedes aegypti is the more productive species as is shown in the comparison of fecundity of the two species regardless of the hosts used. This tendency to produce a great many eggs after blood feeding can be associated with each individual's chances to survive. The larvae of this species are found predominantly in artificial containers containing water and small puddles which can and do frequently dry up desiccating the organisms. Thus, in order to survive, this species must lay a considerably greater number of eggs which have a greater percentage of hatch and very rapid larval development when compared to other species. This survival pattern can be seen in the results.

Anopheles quadrimaculatus larvae are usually found around edges of ponds or lakes. The fact that these mosquitoes are present in much larger bodies of water than *Ae. aegypti* is an indication that this species is not confronted with the same survival problems as *Ae. aegypti*. There is much less chance of a pond drying up as there is water in an artificial container. This means that *An. quadrimaculatus* has a lower mean number of eggs laid and number of eggs hatched regardless of which of the 8 vertebrates was used as the host.

Variation in the size of the female mosquitoes may be

an important reason why I encountered differences in fecundity between the 2 mosquito species and the 8 hosts. Since, for these experiments, I was feeding the larvae a predetermined amount of food each day, and not taking their mortality into account, some of the larvae obtained more food than others thus enabling them to develop into larger adults. Mosquitoes used in each treatment were raised separately. This difference in technique could account for the fact that some adult females of both species took larger blood meals on certain hosts than others. So consequently, the resulting number of eggs laid and per cent viability of eggs could show some variation due to the different sizes of the female mosquitoes.

Despite the results of these experiments with the two mosquitoes, it is possible that these same results may not hold true in different environmental situations or with populations from different areas. An area of North America such as Florida or even another part of Massachusetts might yield populations giving entirely different results when tested for numbers and viability of eggs by An. quadrimaculatus and Ae. aegypti after feeding on the 8 hosts. Possibly the temperature, humidity, time of feeding, length of daylight and other environmental factors would cause these mosquito species to react differently to these same hosts. Also natural hosts of these species from different parts of the country might cause changes in the mosquito's productivity. The results are somewhat dependent on where the culture of mosquitoes was obtained.

It is possible that an evolutionary change could cause different results in these experiments. If the abundance of one common natural host should diminish at any one time, the mosquito would be forced to exist on alternate hosts. If the mosquitoes continually fed on these alternate hosts, the mosquito population would adapt better to this host as a food source.

A mosquito might use a previously poor host for feeding because of a change in the mosquito's nutritional requirements. In pertaining to this experiment, a mutant strain may have caused these mosquitoes to develop a preference for homeotherms over poikilotherms or have caused a development where they prefer birds over mammals as could have happened in the case of Aedes aegypti on quail.

Any changes in the surroundings could cause these mosquitoes to lay a different number of eggs with different numbers hatching when fed on these 8 specific hosts.

Anopheles quadrimaculatus females produced the greatest number of eggs when blood fed on rabbit than on any of the other laboratory animals tested. One possible reason for this result is that the An. quadrimaculatus culture at the University of Massachusetts had been fed previously on rabbits for several months, so the population was gradually selected for host attraction for the rabbit which can be observed by the number of eggs laid and also by per cent hatch of these eggs laid. Those mosquitoes not attracted to the rabbit had been eliminated.

The guinea pig was the second best Anopheline host using number of eggs laid after blood feeding as the criterion. There was a statistically significant difference between the guinea pig and the rabbit as hosts regarding number of eggs produced by the mosquitoes possibly accounted for by the attraction of An. quadrimaculatus to the rabbit. One reason that the guinea pig was such a good host could be that it is closely related phylogenetically to the rabbit. The guinea pig and the rabbit may have more exhaled CO_2 surrounding them, a more attractive body temperature, greater hairiness and more movement than the other hosts. These factors may directly influence the attraction of An. quadrimaculatus to the guinea pig or rabbit.

Of the 2 poikilotherms studied, the turtle was probably superior to the frog in regard to the number of eggs laid by An. quadrimaculatus after having fed on it. As recorded in Table 11, the mean number of eggs laid by An. quadrimaculatus after feeding on the frog is greater than those produced after feeding on the turtle. The frog data are probably biased since only 2 An. quadrimaculatus fed on the frog. Since the turtle was fed on by 10 An. quadrimaculatus this mean is more indicative of the population. The frog and the turtle were heated to 95°F. and 86°F. respectively, to increase their attractiveness to the mosquitoes. The obstacle involved with using the turtle and the frog as hosts for this mosquito was that they had to be heated in a warmed aquarium in order to raise their body temperature. As soon as each of these cold-blooded hosts was removed from the aquarium and placed inside

the mosquito cage, it would rapidly begin to cool to the same temperature as its environment, becoming less attractive to the An. quadrimaculatus. Some other possible reasons why the frog and turtle were not as good hosts for An. quadrimaculatus as the rabbit and guinea pig are that the turtle epidermis may be difficult to penetrate and the skin glands of the frog may make it less attractive to these mosquitoes. Both the frog and the turtle were wet and this could affect the attraction of An. quadrimaculatus and consequently the blood meal size and number of eggs laid.

The mean number of eggs laid by An. quadrimaculatus after having fed on the chicken was less than half the number of eggs laid by An. quadrimaculatus after feeding on the rabbit. Some possible explanations for this result is that since birds are not common hosts of An. quadrimaculatus, the body temperature as well as distinct odor of the chicken and integumentary covering may not be attractive to the mosquitoes and therefore not conducive to proper blood feeding.

The hamster was also a relatively poor host in respect to number of eggs laid by An. quadrimaculatus after feeding on it. The continuous movement of this host did seem to be a possible interference since the An. quadrimaculatus could not maintain a feeding position for any length of time. The size of the hamster was such that it appeared to be less attractive than the larger warm-blooded hosts. The higher body temperature combined with hair texture and less CO_2 exhaled may also have accounted for this result.

The quail was very similar to the chicken regarding the number of eggs laid by An. quadrimaculatus after blood feeding, so the reasons why the quail was such a poor host are similar to those mentioned previously for the chicken.

Anopheles quadrimaculatus laid the fewest number of eggs after feeding on the human host. Possible explanations for this poor result on the human are the lack of (1) hairiness in the host, (2) movement on the part of the host which attracts the mosquitoes, (3) adequate CO_2 to attract An. quadrimaculatus, and (4) the lack of an attracting odor. The mean number of eggs laid by An. quadrimaculatus after feeding on the rabbit was approximately 5 times more than the number of eggs laid by An. quadrimaculatus after feeding on the human host. It is interesting to note than An. quadrimaculatus which fed on the human were completely engorged, according to a visual inspection, yet these individuals laid the fewest number of eggs. Some factor may be lacking in the blood of the human as well as in some or all of the other hosts, which has an effect on the development of eggs within the mosquito.

The eggs laid by An. quadrimaculatus after feeding on the rabbit resulted in the highest number hatched compared to the other hosts. This is good evidence that the rabbit is the best Anopheline host of these 8 laboratory animals tested. A probable explanation for this result is that since the rabbits had been used previously to feed the culture and an attraction to this host was selected for, these mosquitoes

were engorged more than those feeding on the other hosts. Engorgement was determined by visual observation, so there is considerable chance for error here. Some individual mosquitoes may have consumed more blood from one host than other mosquitoes from other hosts. This could then account partially for the different number of eggs laid by An. quadrimaculatus after feeding on the different hosts. Actual weights of the blood volumes should be taken to validate these results further.

The fecundity and egg viability of An. quadrimaculatus after it fed on the guinea pig were considerably less than that of the rabbit, yet noticeably more than any of the other remaining 6 hosts. So again this may allow us to relate the guinea pig more closely to the rabbit using size, body temperature, and body covering as criteria. The guinea pig is definitely the second best Anopheline host in reference to number of eggs laid and hatched after feeding by the mosquito.

The 2 poikilotherms, frog and turtle, and the 2 birds, chicken and quail, were all relatively similar regarding per cent hatch of eggs after having been fed on by the An. quadrimaculatus. As mentioned earlier in this section, these are not common hosts for this mosquito species and there seems to be a lack of attraction by these hosts for this species. This could be demonstrated by a difference in the volume of the blood meal which can reflect on the number of eggs laid and per cent hatch of these eggs.

The eggs laid by An. quadrimaculatus after feeding on

a human resulted in the poorest hatch while the eggs laid by the An. quadrimaculatus after feeding on the hamster showed only a slightly higher mean.

The Anopheline results regarding the human are of interest in that Anopheles quadrimaculatus is a prime vector of malaria in the eastern United States which brings humans into considerable contact with this mosquito, yet according to my results, man is a very poor host. A possible explanation for this difference is that we are dealing with a strain of Anopheles quadrimaculatus which is more attracted to the rabbit or guinea pig because of their odor, color, size, texture of skin, body temperature and CO_2 in the immediate vicinity of the host. Individual variation of the human host may also account for this different result.

Aedes aegypti females laid the greatest mean number of eggs after blood feeding on the quail and chicken. The fact that the quail is a good host can also be seen in Table 8 by the high number of eggs which hatched after Ae. aegypti fed on the quail. A highly probable reason for this result is that the Aedes aegypti culture at the University of Massachusetts was previously fed for many months on quail which could be responsible for a selection of Ae. aegypti for the quail. Other factors which could account for an attraction of Ae. aegypti for the quail and chicken are (1) a favorable host temperature, (2) a greater amount of activity by the host, and (3) a CO_2 production in the vicinity of the host. Ae. aegypti may very well have taken in a

slightly larger blood meal from the birds than from the remaining 6 hosts. This could account for the greater number of eggs laid and hatched. With Ae. aegypti, as with An. quadrimaculatus, the blood volume or weight was not determined. The mosquitoes were only observed visually to have fed to repletion.

Ae. aegypti fed individually on rabbit, guinea pig, and hamster yielded a similar mean number of eggs laid. There was no statistically significant difference between them. This trend among these 3 mammals also carried over into the mean number of eggs hatched after oviposition by Ae. aegypti. Since these 3 mammals are closely related, this is an expected result. Factors which could account for the reason that these were not the best hosts are (1) a less favorable attracting body temperature on the mammals than on the birds, (2) less actual movement of these hosts, (3) a weaker attracting odor, and (4) hairiness of the hosts.

The frog, turtle, and human were all poor hosts of Ae. aegypti in respect to number of eggs laid and hatched after feeding on each host. The warmed frog and the warmed turtle seemed to cool off rapidly when they were placed in the cages after having been heated in water filled aquariums. The convection currents surrounding these hosts as well as the water present on them may be possible reasons for poor attraction of Ae. aegypti. Other factors which may be responsible for a lack of attraction of Ae. aegypti for the frog and turtle are (1) the tough epidermis of the turtle,

(2) the "obnoxious" skin glands of the frog, (3) lack of an attracting odor by the hosts, (4) lack of movement by the hosts, and (5) small amount of CO_2 surrounding the hosts.

The fact that the human was a poor host for Ae. aegypti regarding the number of eggs it laid and their hatchability after feeding is puzzling. Aedes aegypti is so closely associated with yellow fever affecting humans that one would expect a closer host-vector relationship than I found using Ae. aegypti on the human. Possible reasons for these poor results in number of eggs using Ae. aegypti may be due to a (1) lack of an attracting odor to Ae. aegypti, (2) lack of attracting movement, (3) lack of attracting CO_2, and (4) different quality or quantity of hair.

In considering the results obtained by the number of eggs laid and hatched when Ae. aegypti fed on the 8 hosts, we can readily see a division of the hosts according to classes, which is a desirable result since related animals should provide similar results in the same experiments. These results seem to disagree with those of Pena de Grinaldo & LaVoipierre in that there is a significant difference in fecundity between the human and laboratory animals when used as hosts for Aedes aegypti (Table 7).

This experiment should be repeated in an attempt to reduce the possibility of error in results. One could arrive at more precise results by using female mosquitoes of known size as well as determining the amount of host blood engorged. Some of the errors which should be considered are: (1) age of

hosts, (2) health of hosts, (3) the type and area of the surface of the host exposed to the mosquitoes, (4) color of host, (5) time of day to blood feed mosquitoes on hosts, (6) size of mosquitoes, (7) weight of blood meal, (8) greater control over temperature and humidity in lab where actual blood feeding took place, (9) actual movement of the hosts during feeding of mosquitoes, (10) the type of cage used to retain adults before feeding.

CONCLUSIONS

The following conclusions may be made according to these studies:

(1) Aedes aegypti exhibits a definite pattern of fecundity and egg viability after feeding on different types of hosts. A grouping according to classes of vertebrate hosts was observed. The exception to this is the number of eggs laid after Ae. aegypti fed on the human host. No such pattern was exhibited by An. quadrimaculatus.

(2) Aedes aegypti was more fecund and produced more viable eggs after feeding on avian hosts, whereas Anopheles quadrimaculatus did best after feeding on the lagomorph and did very poorly on the avians.

(3) Fecundity and egg viability of Aedes aegypti were similar when the lagomorph and rodents were used.

(4) Aedes aegypti exhibited poor fecundity and egg viability after feeding on the poikilotherms even though their body temperatures were raised to approximately that of homeotherms. Therefore, heat, movement, and CO_2 production do not seem to be the sole stimuli for feeding.

(5) The human was a poor host of both Aedes aegypti and Anopheles quadrimaculatus in respect to fecundity and egg viability.

(6) The frog proved to be a host of poor acceptability. This is based on a small number of feedings. However, Anophelines that did feed produced viable eggs.

(7) The fecundity of Anopheles quadrimaculatus was greater after feeding on the poikilotherms than after feeding on either the avian or some mammals.

SUMMARY

A group of experiments was conducted using 2 mosquito species, Aedes aegypti (L.) and Anopheles quadrimaculatus Say, and 8 vertebrate hosts including rabbit, quail, chicken, guinea pig, hamster, turtle, frog, and human, in an attempt to obtain information on the suitability of these hosts for the 2 mosquito species. Number of eggs laid and per cent eggs hatched were used as the criteria for evaluation. The raw data, which are number of eggs laid and per cent of eggs hatched, were programmed through a computer to provide an analysis of variance. The Duncan's Multiple Range Test was performed to determine significant differences among the 8 hosts in respect to number of eggs laid and hatched for these 2 mosquitoes when blood fed on these hosts.

The rabbit proved to be the best host when compared to the other hosts for Anopheles quadrimaculatus using number of eggs laid and hatched after blood feeding as criteria.

The quail and the chicken were the best hosts of Aedes aegypti using number of eggs laid and per cent hatch of these eggs as criteria.

This experiment should be repeated in an attempt to reduce the amount of experimental error which presently exists. Many of the error factors have been listed in the discussion.

LITERATURE CITED

Bates, M. 1940. The nomenclature and taxonomic status of the mosquitoes of the Anopheles maculipennis complex. Ann. Ent. Soc. Amer. 33: 343-353.

_____. 1965. The natural history of mosquitoes. New York: Harper & Row. 378 p.

Bishop, A., and B. M. Gilchrist. 1946. Experiments upon the feeding of Aedes aegypti (L.) through animal membranes, etc. Parasitology 37: 85-100.

Brown, A. W. A. 1951. Studies of the responses of the female Aedes mosquito. Part IV. Field experiments on Canadian species. Bull. Ent. Res. 42: 575-582.

_____, D. S. Sarkaria and R. P. Thompson. 1951. Studies on the responses of the female Aedes mosquito. Part I. The search for attractant vapors. Bull. Ent. Res. 42: 105-114.

Bull, C. G., and F. M. Root. 1923. Preferential feeding experiments with Anopheline mosquitoes. Amer. J. Hyg. 3: 514-520.

Burgess, R. W., and M. D. Young. 1944. Methods of handling and feeding Anopheles quadrimaculatus Say upon malarious patients. J. Nat. Malar. Soc. 3: 241-247.

Chapman, H. C., and D. B. Woodward. 1965. Blood feeding and oviposition of some flood water mosquitoes in Louisiana: Laboratory studies. Mosq. News 25: 259-262.

Christophers, S. R. 1960. Aedes aegypti (L.). The yellow fever mosquito: Its life history, bionomics and structure. London: Cambridge Univ. Press. 739 p.

Clements, A. N. 1963. The physiology of mosquitoes. New York: Macmillan. 393 p.

Colless, D. H., and W. T. Chelapah. 1960. Effects of body weight and size of blood meal upon egg production in Aedes aegypti (L.) (Diptera: Culicidae). Ann. Trop. Med. Parasit. 54: 475-482.

De Foliart, G. R. 1967. Aedes canadensis (Theobald), Feeding on Blanding's turtle. J. Med. Ent. 4: 31.

Downe, A. E. R. 1960. Blood meal sources and notes on host preferences on some Aedes mosquitoes (Diptera: Culicidae). Can. J. of Zool. 38: 689-699.

Edman, J. D., and A. E. R. Downe. 1964. Host blood sources and multiple feeding habits of mosquitoes in Kansas Mosq. News 24: 154-160.

Fielding, J. W. 1919. Notes on the bionomics of Stegomyia fasciata F. Ann. Trop. Med. Parasit. 13: 259-296.

Geoffrey, G. M. 1956. Blood meal volume in Anopheles quadrimaculatus, An. albimanus and Aedes aegypti. Exp. Parasit. 5: 371-375.

Gordon, R. M., and W. H. R. Lumsden. 1939. A study of the behavior of the mouthparts of mosquitoes when taking up blood from living tissue; together with some observations on the ingestion of microfilariae. Ann. Trop. Med. Parasit. 33: 259-278.

Horsfall, W. R. 1955. Mosquitoes: their bionomics and relation to disease. New York: Ronald Press. 723 p.

Howard, L. O., H. G. Dyar and F. Knab. 1912. The mosquitoes of North and Central America and the West Indies. Washington: Carnegie Institute. Vol. I. 520 p.

Keener, G. G., Jr. 1945. Detailed observations on the life history of Anopheles quadrimaculatus Say. J. Nat. Malar. Soc. 4: 263-270.

Kennedy, J. S. 1939. The visual responses of flying mosquitoes. Proc. Zool. Soc. London 109: 221-242.

King, W. V., and C. G. Bull. 1923. The blood feeding habits of malaria carrying mosquitoes. Amer. J. Hyg. 3: 497-513.

Laarman, J. J. 1958. The host seeking behavior of Anopheline mosquitoes. Trop. and Geogr. Med. 10: 293-305.

Lumsden, W. H. R. 1947. Observations on the effect of microclimate on biting by Aedes aegypti (L.) (Diptera: Culicidae). J. Exp. Biol. 24: 361-373.

Mathis, M. 1934. Agressivité et pontes comparées du moustique de la fièvre jaune en conditions expérimentales. Comptes Rendus des Séances de la Société de Biologie 115: 1624-1626.

Mayne, B. 1928. A note on some recent attempts to transmit malaria organisms mechanically through mosquito biting. Indian J. Med. Res. 15: 1067-1071.

Murphey, F. J., P. P. Burbutis and D. F. Bray. 1967. Bionomics of Culex salinarius Coquillett II. Host acceptance and feeding by adult females of C. salinarius and other mosquito species. Mosq. News 27: 366-374.

Nolan, M. P., Jr., M. A. Moussa and D. E. Hayes. 1965. Aedes mosquitoes feeding on turtles in nature. Mosq. News 25: 218-219.

Nuttall, G. H. F., and A. E. Shipley. 1902. Studies in relation to malaria. II. The structure and biology of Anopheles. (Anopheles maculipennis). J. Hyg. 2: 58-84.

Parker, A. H. 1948. Stimuli involved in the attraction of Aedes aegypti (L.) to man. Bull. Ent. Res. 39: 387-397.

Pearson, W. G., and B. A. Harrison. 1967. Research notes on Aedes aegypti feeding on the eastern garter snake Thamnophis sirtalis. Mosq. News 27: 199.

Pena de Grimaldo, E., and M. M. J. LaVoipierre. 1960. Effectos comparativos de la sangre humana como fuente de alimentacion de los mosquitos Aedes aegypti variedad queenslandensis con la sangre de los animales de laboratorio, usando el numero de oocitos desarollados despues de una sola ingestion de sangre como criterio. Rev. Iber. Parasit. Granada 20: 23-30.

Peterson, D. G., and A. W. A. Brown. 1951. Studies on the responses of the female Aedes mosquito. Part III. The responses of Aedes aegypti (L.) to a warm body and its radiation. Bull. Ent. Res. 42: 535-541.

Reid, J. A. 1961. The attraction of mosquitoes by human or animal baits in relation to the transmission of disease. Bull. Ent. Res. 52: 43-62.

Roth, L. M. 1951. Loci of sensory end organs used by mosquitoes (Aedes aegypti (L.) and Anopheles quadrimaculatus Say) in receiving host stimuli. Ann. Ent. Soc. Amer.

44: 59-74.

Roubaud, E. 1934. Observations sur la fecondité de Anophelines. Bull. Soc. Path. exot. 27: 853-854.

Roy, D. N. 1931. On the ovulation of A. stephensi. Indian J. Med. Res. 19: 629-634.

Shannon, R. C., and J. Hadjinicalao. 1941. Egg production of Greek Anophelines in nature. J. Econ. Ent. 34: 300-305.

Sippell, W. L., and A. W. A. Brown. 1953. Studies of the responses of the female Aedes mosquito. Part V. The role of visual factors. Bull. Ent. Res. 43: 567-574.

Smart, M. R., and A. W. A. Brown. 1956. Studies on the responses of the female Aedes mosquito. Part VII. The effect of skin temperature, hue and moisture on the attractiveness of the human hand. Bull. Ent. Res. 47: 89-100.

Smith, A. 1955. On the transmission of bancroftial filariasis on Ukara Island, Tanganyika. IV. Host preferences of mosquitoes and the incrimination of Anopheles gambiae Giles and An. funestus Giles as vectors of bancroftial filariasis. Bull. Ent. Res. 39: 387-397.

Terzian, L. A., and N. Stahler. 1949. The effects of larval population density on some laboratory characteristics of Anopheles quadrimaculatus Say. J. Parasit. 35: 487-495.

Toumanoff, C. 1949. L'hemophagie variée et l'activité reproductrice chez Aedes aegypti (L.) et Aedes albopictus Skuse. Bull. Soc. Path. exot. 42: 466-470.

Van Thiel, P. H. 1937. Quelles sont les excitations incitant l'Anopheles maculipennis atroparvus à visiter et à piquer l'homme ou le bétail? Bull. Soc. Path. exot. 30: 193-203.

Wardle, R. A. 1929. The problems of applied entomology. New York: McGraw-Hill Book Company, Inc. 587 p.

Willis, E. R. 1958. The use of artificial heat to induce mosquitoes to feed on cold-blooded animals. Ann. Ent. Soc. Amer. 51: 257-261.

Woke, P. A. 1937a. Cold blooded vertebrates as hosts for Aedes aegypti (L.). J. Parasit. 23: 310-311.

_____. 1937b. Comparative effects of the blood of different species of vertebrates on egg production of Aedes aegypti (L.). Amer. J. Trop. Med. 17: 729-745.

_____, M. S. Ally and C. R. Rosenberger. 1956. The number of eggs developed related to the quantities of human blood ingested in Aedes aegypti (L.) (Diptera: Culicidae). Ann. Ent. Soc. Amer. 49: 435-441.

APPENDIX

TABLE 7.--Fecundity and egg viability of Aedes aegypti when fed on various mammals

	Hosts							
	Rabbit		Guinea Pig		Hamster		Human	
	A	B	A	B	A	B	A	B
Rep. 1	114	0	112	95	121	77	84	83
2	116	95	103	94	65	38	95	97
3	100	45	125	88	80	48	100	85
4	118	36	93	96	78	86	75	77
5	104	85	80	93	78	74	77	82
6	108	44	78	0	112	87	81	58
7	108	60	91	0	125	70	84	93
8	125	81	82	95	130	71	73	73
9	93	97	126	41	84	68	64	45
10	98	54	107	0	125	88	74	34
11	126	70	75	0	130	93	78	65
12	137	77	95	0	117	79	78	78
13	101	61	129	75	106	91	71	68
14	87	61	100	0	134	92	96	81
15	127	70	67	0	56	47	36	75
16	68	64	126	0	43	0	79	91
17	95	92	81	0	94	23	67	70
18	45	67	108	85	64	50	71	0
19	82	91	105	83	125	55	94	88
20	114	0			96	75	54	69
21	112	0			114	80	40	0
22	100	68			87	33	84	0
23					123	86	80	90

A = number of eggs laid

B = per cent hatched

TABLE 8.--Fecundity and egg viability of *Aedes aegypti* when fed on various avian hosts

	Chicken		Quail			Chicken		Quail	
	A	B	A	B		A	B	A	B
Rep. 1	118	70	121	89	Rep. 26	118	77	134	84
2	141	69	128	39	27	123	83	135	79
3	109	74	133	82	28	114	73	114	82
4	110	65	141	88	29	105	75	122	75
5	114	59	118	83	30	119	72	127	66
6	9	0	145	78	31	121	66		
7	154	83	138	67	32	112	72		
8	25	0	154	75	33	111	65		
9	27	0	79	61	34	100	74		
10	111	76	119	66	35	99	64		
11	102	75	149	81	36	31	13		
12	125	82	115	81	37	109	63		
13	105	70	71	38	38	112	64		
14	111	78	119	78	39	98	58		
15	22	0	147	80	40	105	69		
16	108	73	111	66	41	122	70		
17	37	24	162	78	42	89	64		
18	141	84	111	74	43	100	76		
19	120	81	134	84	44	114	73		
20	81	60	105	78	45	120	72		
21	140	83	117	78	46	75	41		
22	117	86	122	69	47	122	0		
23	125	90	141	83	48	97	0		
24	119	77	139	77					
25	64	64	139	80					

A = number of eggs laid
B = per cent hatched

TABLE 9.--Fecundity and egg viability of Aedes aegypti fed on poikilothermic vertebrate hosts

	Turtle		Frog	
	A	B	A	B
Rep. 1	102	85	5	0
2	70	90	116	84
3	100	71	101	82
4	68	0	21	76
5	80	80	91	79
6	92	88	17	82
7	71	92	91	13
8	64	53	110	88
9	82	82	86	84
10	87	80	67	76
11	56	93	35	77
12	85	49	71	85
13	62	76	97	66
14	65	58	63	79
15	80	6		
16	49	34		
17	90	76		
18	64	73		
19	67	78		

A = number of eggs laid
B = per cent hatched

TABLE 10.--Fecundity and egg viability of *Anopheles quadrimaculatus* fed on various mammal hosts

	Hosts							
	1 Rabbit		2 Guinea Pig		3 Hamster		4 Human	
	A	B	A	B	A	B	A	B
Rep. 1	166	92	156	92	82	89	80	90
2	29	72	125	90	12	8	10	60
3	160	94	69	71	127	69	5	80
4	101	94	104	92	130	83	17	76
5	139	95	115	76	120	0	80	8
6	158	86	93	97	63	8	7	71
7	95	65	158	83	34	76	13	92
8	132	92	51	59	72	58	2	50
9	167	88	26	42	8	13	55	0
10	145	90	142	78	8	13	11	64
11	126	84	74	78	50	86	2	100
12	136	83	101	91			9	11
13	187	76	83	94			7	14
14	149	85	106	90			3	0
15	163	85					83	89
16	190	94					42	76
17	137	88						

A = number of eggs laid
B = per cent hatched

TABLE 11.--Fecundity and egg viability of Anopheles quadrimaculatus fed on avian and poikilothermic vertebrate hosts

	Hosts							
	5 Chicken		6 Quail		7 Turtle		8 Frog	
	A	B	A	B	A	B	A	B
Rep. 1	127	94	36	56	95	85	94	95
2	77	9	115	74	87	71	72	15
3	125	70	39	69	81	90		
4	68	56	59	73	78	88		
5	96	23	78	81	62	92		
6	143	17	92	88	76	83		
7	21	0	51	92	91	81		
8	51	37	39	69	59	0		
9	10	20	65	88	83	92		
10	57	93	96	91	68	82		
11	20	20	23	87				
12	7	0	75	88				
13	7	71	93	86				
14	97	88	76	70				
15	136	76	58	67				
16	65	91						
17	26	58						
18	6	0						
19	93	86						

A = number of eggs laid
B = per cent hatched

TABLE 12.--Trials used to determine the average amount of food given in grams per Anopheles quadrimaculatus larvae with the small and large feeding devices

	Small Measure	Large Measure
Trial 1.	.003	.017
2.	.003	.052
3.	.003	.046
4.	.003	.048
5.	.005	.053
6.	.003	.054
7.	.003	.052
8.	.003	.048
9.	.005	.046
10.	.004	.053
11.	.004	.060
12.	.005	.046
13.	.005	.049
14.	.005	.054
15.	.004	.043
16.	.004	.049
17.	.004	.047
18.	.006	.059
19.	.005	.054
20.	.006	.052
$\bar{x}$ =	.004	.049